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Mouthwash in the etiology of oral cancer in Puerto Rico

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Abstract

Objectives: To determine if the risk of cancers of the mouth and pharynx is associated with mouthwash use in Puerto Rico, an area of relatively high risk.
Methods: Interviews were conducted with 342 cases of oral and pharyngeal cancer registered in Puerto Rico and diagnosed between 1992 and 1995 and with 521 population-based controls regarding mouthwash use and other factors. Mouthwash-related risks were estimated using unconditional logistic regression controlling for potential confounders.
Results: The adjusted odds ratio associated with using mouthwash with an alcohol content of 25% or greater was 1.0. Risks were not higher with greater frequency, years of use, or lifetime mouthwash exposure. Among tobacco and alcohol abstainers the odds ratio associated with mouthwash use was 2.8 (CI = 0.8–9.9), in contrast to 0.8 (CI = 0.4–1.7) and 0.9 (CI = 0.6–1.3) among those with light and heavy cigarette smoking/alcohol drinking behaviors, respectively.
Conclusions: There was no overall increased risk of oral cancer associated with mouthwash use. An elevated, but not statistically significant, risk was observed among the small number of subjects who neither smoked cigarettes nor drank alcohol, among whom an effect of alcohol-containing mouthwash would be most likely evident. Our findings indicate the need to clarify the mechanisms of oral carcinogenesis, including the possible role of alcohol-containing mouthwash.

Introduction

In many countries worldwide, a substantial percentage of oral and pharyngeal cancers (hereafter referred to as oral cancer) is attributable to tobacco use and alcohol drinking [1–3]. However, oral cancer can occur in the absence of tobacco and alcohol use [4, 5]. For example, in a study in Puerto Rico, where the rates are higher than in many areas of the western hemisphere [6], we found that a quarter of the oral cancers among men and half of the cancers among women were not attributable to tobacco use and alcohol drinking [7]. To explain the occurrence of oral cancer among tobacco and alcohol abstainers, attention has been drawn to other potential risk factors, including mouthwash use [8], oral cavity infections and diseases, denture sores, poor oral hygiene and poor dentition [9–14], dietary insufficiencies, especially of fruits and vegetables [15–17], and low levels of serum nutrients such as carotenoids [18]. Also under study is the possible role of human papillomaviruses in the etiology of oral cancer [19].

Mouthwash use has been the subject of several epidemiologic studies of oral cancer. In a large population-based case–control study in the United States, we found that mouthwash use was associated with a 40% excess risk of oral cancer among males and 60% among females, after correcting for the effects of tobacco use and alcohol drinking [8]. Risks increased with both

frequency and duration of use; persons using these products 60 or more times per month had 1.7–2.0-fold increased risks of oral cancer, while users for 40 years or more had 1.4–1.9-fold increased risks. In other studies of oral cancer the association with mouthwash use has been inconsistent, with odds ratios ranging from 0.8 to 2.5 [20–26]. Some concerns have been raised about the potential for misclassification of tobacco and alcohol use, which might lead to spuriously elevated risks among mouthwash users [27].

In the US population-based study of mouthwash use and oral cancer [8], elevated risks were confined to users of mouthwash containing high concentrations of alcohol (≥25%). A causal relationship seemed plausible, since alcoholic beverage consumption is a strong independent risk factor for oral cancer, based on epidemiologic studies in many populations around the world [28]. Although systemic effects may contribute to alcohol-associated oral cancer [29], the mechanism seems likely to involve direct contact of alcohol with the oral mucosa, whether the vehicle is an alcoholic beverage or mouthwash, which is generally expectorated and not swallowed. The local effects of alcohol may result from its metabolism to acetaldehyde [30] in oral epithelium or by oral microbes [31] or from the increased permeability of the oral mucosa to tobacco and other carcinogens [32]. The role of acetaldehyde in oral carcinogenesis is supported by the excess risk that we found among heavy drinkers in this study population who have the fast-metabolizing genotype at the alcohol dehydrogenase type 3 locus [33].

This analysis evaluates the role of alcohol-containing mouthwash in the etiology of oral cancer, with special attention to the risk among non-users or light users of tobacco and alcoholic beverages, a subgroup in which the impact of mouthwash would likely be most evident.

Methods

Detailed methods for the case–control study of oral cancer in Puerto Rico are provided elsewhere [7]. Eligible for the study were all persons, aged 21–79 years, diagnosed in Puerto Rico with a newly incident, histologically confirmed cancer of the oral cavity (excluding lip) or pharynx (excluding nasopharynx) (*International Classification of Diseases for Oncology* codes C01–C14) [34] between December 1992 and February 1995 and a sample of population-based controls, frequency-matched by age and gender. No information is available on the completeness of the registry. Controls under age 65 were selected from a two-stage area probability sampling frame, involving randomly selecting with probability proportionate to size municipios (similar to counties) in the first stage and segments (blocks or combinations of blocks) in the second stage. All dwelling units in each segment were listed, and samples were selected separately for male-and female-designated households. Older controls (ages 65 and older) were selected by systematic sampling from rosters of Medicare enrollees. Trained interviewers conducted in-person, structured interviews with study subjects in Spanish. Respondents provided information on their customary use of tobacco and alcohol, medical and oral health history, numbers of sexual partners, diet, and occupation. Biological specimens (tumor tissue from cases only, blood, urine, and oral epithelial cell washings) were obtained from selected case and control subjects.

Of the 519 eligible cases and 629 eligible controls, 367 (71%) of the cases and 521 (83%) of the controls were interviewed. A total of 342 cases were not of salivary gland origin. This group was analyzed separately from the 25 cases with salivary gland cancers, which also included cases who had oral tumors whose histologic types suggested a salivary gland origin [7].

Whether subjects had ever used mouthwash was determined by their response to the following question: "Now I'd like to ask about mouthwash use. Before one year ago, did (you/subject's name) ever use mouthwash on a regular basis? By regular basis we mean at least once a week for six months or more." The reference period of "before one year ago" was used because the interviews took place several months after the cases' initial diagnosis of cancer, and the reference period was intended to focus on experiences prior to this event and to exclude changes in habits related to symptomatic disease. Questions about mouthwash use were very similar to those used in a previous study [8].

The questionnaire elicited information about the type of mouthwash used, including specific mouthwash brand names, use of hydrogen peroxide and fluoride rinses [no subjects reported use of fluoride rinses), and any other forms of mouthrinse. Brands were classified by alcohol content as "high" (25% or more), "low" (0.1–24.9%), or no alcohol content based on the 1994 *Physician's Desk Reference for Nonprescription Drugs* [35]. If alcohol concentration information was unavailable from the *Physician's Desk Reference*, then the following sources were consulted in turn: an article on mouthwashes in *Consumer Reports* [36] and ingredient labels on mouthwash products. Information on the frequency and duration of use was obtained, as well as other characteristics of use, such as whether the mouthwash was used diluted or full-strength. The variable "mouthwash-years" combined frequency and duration

of use, specifically the daily frequency of use was multiplied by years of use.

Persons were classified as cigarette smokers if they had smoked at least 100 cigarettes in their life. Years smoked cigarettes took into account use and quit periods. The usual number of packs smoked per day times the number of years smoked times 365 equaled lifetime number of packs smoked. Persons were considered to be cigar, pipe, chewing tobacco, and snuff users if they had used these products for at least 6 months. Alcohol drinkers were persons who had at least 12 drinks in a lifetime. Subjects were permitted to express the amounts consumed in the units of their choice; this amount was then converted to common units. A drink was defined as 4 ounces of wine, 1½ ounces of hard liquor, or 12 ounces of beer. The number of lifetime drinks was determined by multiplying the usual amount drunk per week by the number of years alcohol was consumed by 52.

Users of tobacco or alcohol were grouped into light and heavy categories. The light smoker and alcohol drinker category included smokers of less than 10 cigarettes per day who consumed six or fewer alcoholic beverage drinks per week. Heavy cigarette smokers and alcohol drinkers smoked 10 or more cigarettes per day regardless of their drinking status or drank more than seven drinks per week regardless of their cigarette smoking status. Alcohol dehydrogenase 3 genotypes were determined using methods described elsewhere [33].

The use of mouthwash by controls was examined according to behavioral and demographic characteristics to understand patterns of mouthwash use and to aid in the selection of variables for the multivariate models. The Mantel–Haenszel chi-square statistic [37, 38] was used to assess among controls the linear association between ordinal behavioral and demographic variables and the use of mouthwash. In the computation of this statistic, levels of each ordinal classification variable received scores of 0, 1, 2, *etc*., and mouthwash use was coded as one if used and zero if not used. ANOVA tests [39] were used to evaluate heterogeneity in the mean years of mouthwash use or the mean frequency of use per day within categories of age, education, income, tobacco use, alcoholic beverage consumption, tooth-brushing habits, and dental care visits.

Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated by unconditional logistic regression analysis [40]. Control variables in the models for males and for both genders combined were as follows: age (less than 54 years, 5-year intervals from 55 to 69, 70 years and older); tobacco use expressed as lifetime number of cigarette packs (no tobacco use, 1–5000, 5001–10,000, 10,001–20,000, 20,001 or more packs, and no cigarettes but use of other tobacco); alcoholic beverage consumption expressed as lifetime number of drinks (no alcohol, 1–10,000, 10,001–40,000, 40,001–80,000, 80,001 or more drinks); and consumption of raw fruits and vegetables in quartiles of the control distribution. The combined models also included a term for gender. The models for females differed in that age was classified as ages less than 55, 55–69, and 70 and older, and tobacco and alcohol drinking variables were grouped into fewer categories due to the smaller numbers of women and their lower use of these products. For female-specific models the lifetime tobacco use categories were: no tobacco use, 1–10,000 and 10,001 or more cigarette packs, and no cigarettes but use other tobacco. The lifetime alcohol use categories were: no alcohol, 1–1000, and 1001 or more drinks. All ORs were adjusted unless otherwise noted.

To address the potential for confounding by low socioeconomic status [41], additional models also included education (less than high school, graduated from high school, and more than high school) or current household income (less than $10,000, $10,000–14,999, $15,000–19,999, and $20,000 or more). Results from models containing these variables were not substantially different from models without these variables, so the findings from these more complex models are not presented here.

To test for trend, the exposure variables – number of times per day mouthwash was used, years used, and mouthwash-years – were treated as continuous in the model with each level of the categorical variable represented by the mean value of that category among the controls. The values 0 (least mouthwash exposure) to 3 (most mouthwash exposure) were used for the other (ordinal) variables, which are shown in the tables with the least exposed category first and the greatest exposed category last.

Results

Forty-one percent of controls reported having used mouthwash, with similar percentages of male (40.3%) and female (44.2%) users (Table 1). Among male controls, mouthwash use tended to be more common among younger men. Male users were more likely to have had higher education and incomes, more frequent tooth-brushing, and regular dental visits. Mouthwash use tended to be more common among males who smoked the greatest number of cigarettes per day; in contrast, non-drinkers and light alcohol drinkers were more likely to use mouthwash than heavy drinkers. Patterns for females were less clear, perhaps due to small numbers and a limited range of alcoholic beverage

Table 1. Characteristics of mouthwash use among control subjects

	Mouthwash use							
	Males				Females			
	No.[a]	Percentage used[b]	Years used[c,d]	Times/day used[c,d]	No.[a]	Percentage used[b]	Years used[c,d]	Times/day used[c,d]
All controls	417	40.3	21	1.8	104	44.2	21	1.8
Age (years)								
< 54	105	46.7	16	1.8	35	45.7	16	1.9
55–69	208	39.4	23	1.8	38	44.7	26	2.1
70 or older	104	35.6	25	1.7	31	41.9	21	1.3
p-Value		0.08	0.01	0.61		0.76	0.18	0.33
Education								
Less than high school	245	34.7	20	1.8	61	47.5	21	1.5
Graduated high school	85	38.8	23	1.8	16	31.3	17	3.2
More than high school	87	57.5	21	1.8	26	42.3	19	1.9
p-Value		0.0006	0.68	0.96		0.53	0.80	0.05
Average annual income								
≤$9999	170	33.5[c]	21	1.7	63	39.7	22	1.7
$10,000–14,999	114	40.4	22	1.8	14	42.9	20	1.7
$15,000–19,999	53	49.1	18	1.8	12	66.7	18	2.5
$20,000 or more	63	50.8	20	1.9	12	41.7	23	1.5
p-Value		0.008	0.72	0.88		0.37	0.91	0.53
No. cigarettes per day								
None	140	38.6	25	2.1	71	50.7	21	1.9
0–9	61	39.3	22	1.6	9	22.2	*	*
10–19	53	41.5	13	1.4	7	14.3	*	*
20–39	89	39.3	19	1.4	12	41.7	*	1.2
40 or more	65	49.2	23	1.9	2	50.0	*	*
Other tobacco use only	9	11.1	*	*	3	33.3	*	*
p-Value		0.23[e]	0.05	0.03		0.23[e]	0.34	0.95
No. alcoholic beverage drinks per week								
0	67	50.8	26	2.2	70	45.7	20	2.0
1–7	117	42.7	20	1.6	30	40.0	23	1.3
8–21	87	40.2	19	1.5	1	0	–	–
22–42	55	38.2	15	2.0	1	0	–	–
43 or more	58	31.0	24	1.9	0	–	–	–
p-Value		0.03	0.11	0.15		0.26	0.68	0.21
No. times brush teeth per week								
< 13	82	32.9	21	1.9	9	11.1	*	*
14–20	250	39.2	21	1.6	56	42.9	25	1.7
> 20	84	50.0	22	2.1	39	53.9	18	2.0
p-Value		0.02	0.92	0.09		0.03	0.21	0.42
Ever visited dentist on regular basis								
Yes	61	54.1	23	2.0	31	54.8	21	2.1
No	350	38.3	21	1.7	71	40.9	21	1.6
Never been	6	16.7	*	*	2	0.0	*	*
p-Value		0.01	0.45	0.58		0.09	0.99	0.32

* Fewer than five subjects in cell.
[a] May not add to total number of persons due to missing responses to questions.
[b] Mantel–Haenszel chi-square test was used for proportions.
[c] Years used and frequency of use are among mouthwash users only.
[d] *p*-Value based on the *F*-statistic for means.
[e] Other tobacco users not included in computation of the Mantel–Haenzel chi-square statistic.

intake, but, as for males, mouthwash use was associated with more frequent tooth-brushing. Also, the direction of the trends by age and by dental visits paralleled those for males. Among users of mouthwash, men and women reported similar duration (mean of 21 years) and frequency of use (1.8 times per day). Within the

subgroups shown in Table 1, there was little heterogeneity in duration or frequency of use except that the mean years of mouthwash use was higher at older ages.

Of the 342 non-salivary cases, 33.9% of 286 males and 46.4% of 56 females used mouthwash. Risks were not elevated for those who initiated use of mouthwash within 4 years of the interview (crude OR for males = 1.0, CI = 0.4–2.4, based on nine cases and 12 controls; OR for females = 1.0, CI = 0.2–4.1, based on three cases and six controls). To exclude the possibility that mouthwash use was initiated because of oral cancer symptoms before diagnosis, the cases and controls who recently initiated mouthwash use (less than 4 years ago) were excluded from further analysis.

Nearly all mouthwash users used brands containing 25% alcohol or more. Only two cases and six controls exclusively used mouthwash containing 0.1–24.9% alcohol. Because of the small numbers of persons in this subset, the data on these subjects were not analyzed further. Two cases and one control used only alcohol-free mouthwash and were classified in Tables 2–4 as non-users of mouthwash. One control who used mouthwash with unknown alcohol content was excluded from further analysis. In addition, rum was used reportedly as a mouthwash by seven study subjects, but six of them also used other forms of mouthwash, and this use of rum is not considered further. Thus, in Tables 2–4, users of mouthwash were defined as persons using mouthwash containing 25% alcohol or more, while non-users were those who never used mouthwash or used only non-alcohol-containing mouthwash.

Based on the remaining 328 cases and 496 controls, the overall adjusted odds ratio for ever use of mouthwash containing 25% or more alcohol was 1.0 (CI = 0.7–1.4), close to the crude odds ratio of 0.8 (CI = 0.6–1.1). As shown in Table 2, the risk for females (OR = 2.1, CI = 0.9–5.0) exceeded that for males (OR = 0.8, CI = 0.5–1.2), but both confidence intervals included the value 1.0. To determine if tighter control of confounding changes the magnitude of the odds ratio, the model for mouthwash use for females was recalculated replacing the broader variables for females with the tighter ones used in analyses of males and both genders. Using this strategy the odds ratio was 2.9, higher than 2.1 for the model with broader variables.

Among both genders combined and each gender separately, no consistent pattern of risk was associated with the number of times mouthwash was used per day, the number of years used, mouthwash years, or other characteristics of use including the amount of time mouthwash was kept in the mouth or whether the subjects gargled or rinsed the mouth after use. Among females there was a significantly elevated OR of 2.7 (CI = 1.1–7.0) for using diluted mouthwash and a marginally significantly elevated OR of 2.9 (CI = 1.0–8.5) for females using mouthwash less than twice per day. Most users were still using mouthwash in the year prior to the interview. No excess risk was evident in current mouthwash users, but the risks for persons quitting within the past 1–3 years or 4 or more years were 3.8 (CI = 1.3–10.7) and 2.5 (CI = 0.9–7.0), respectively, for both genders combined. All users expectorated the mouthwash except for two cases and two controls who reported swallowing the mouthwash. The reason given for using mouthwash was "personal" for nearly all study subjects. "Medical" use only was the reason given for only four cases and 15 controls (OR = 0.7, CI = 0.2–2.8).

In Table 3, risks are shown for oral *vs.* pharyngeal cancer. Odds ratios associated with mouthwash use were slightly higher for oral than pharyngeal cancer, but no overall association with mouthwash use was seen for either the oral (OR = 1.0, CI = 0.7–1.8) or pharyngeal sites (OR = 0.7, CI = 0.4–1.3). Examination by anatomic subsite yielded non-significant odds ratios for cancers of the palate (OR = 0.4, CI = 0.2–1.0), hypopharynx (OR = 0.6, CI = 0.3–1.4), oropharynx (OR = 0.7, CI = 0.3–1.5), base of tongue (OR = 1.6, CI = 0.8–3.6), other parts of the tongue (OR = 1.4, 0.7–3.1), floor of mouth (OR = 1.4, CI = 0.6–3.2), and other mouth subsites (OR = 1.4, 0.3–3.5).

Table 4 shows odds ratios for mouthwash use according to levels of cigarette smoking and alcoholic beverage consumption adjusting for age, gender, and raw fruits and vegetables. Subjects who used only other tobacco products were excluded from this analysis. Among non-smokers who did not use alcohol (abstainers), the odds ratio for ever use of mouthwash was 2.8, but the confidence interval included 1.0 (CI = 0.8–9.9). The risk among light cigarette smokers who were also light alcohol drinkers (OR = 0.8, CI = 0.4–1.7) was similar to that for heavy smokers and drinkers (OR = 0.9, CI = 0.6–1.3). Among persons who used alcohol but not cigarettes, risks were 1.5 (CI = 0.3–7.4); non-drinkers who smoked also had an elevated but non-significant OR (OR = 1.9 (0.3–13.6)). Risks associated with mouthwash use were significantly or marginally significantly elevated among tobacco and alcohol abstainers who used mouthwash less than 20 years (OR = 6.1, CI = 1.1–33.9), had 30 or more mouthwash years of exposure (OR = 5.1, CI = 1.0–26.0), diluted the mouthwash (OR = 4.1, CI = 1.0–16.4), or just swished the mouthwash in the mouth (OR = 5.4, CI = 1.2–23.9). Excess risks also were found for light smokers and drinkers who stopped use of mouthwash

Table 2. Adjusted[a] odds ratios for use of mouthwash containing an alcohol concentration of 25% or more, non-salivary gland cancers

Mouthwash use	Males		Females		Both genders
	No. of cases/ controls[b]	OR (95% CI)[c]	No. of cases/ controls[b]	OR (95% CI)[c]	OR (95% CI)[c]
Ever used					
No	189/249	1.0 (referent)	30/58	1.0 (referent)	1.0 (referent)
Yes	87/152	0.8 (0.5–1.2)	22/37	2.1 (0.9–5.0)	1.0 (0.7–1.4)
No. times per day					
Less than 2	43/67	0.8 (0.4–1.4)	13/20	2.9 (1.0–8.5)	1.1 (0.7–1.8)
2 or more	44/84	0.7 (0.4–1.3)	9/17	1.5 (0.5–4.5)	0.9 (0.5–1.4)
p-Value for trend test		0.25		0.25	0.71
Years used					
Less than 20	38/72	0.6 (0.3–1.0)	9/17	2.2 (0.7–7.2)	0.7 (0.4–1.2)
20 or more	47/69	1.1 (0.6–1.9)	10/17	1.8 (0.6–5.2)	1.3 (0.8–2.2)
p-Value for trend test		0.97		0.27	0.41
Mouthwash-years					
1–29	40/72	0.6 (0.3–1.1)	10/16	2.5 (0.8–8.0)	0.8 (0.5–1.4)
30 or more	41/70	1.0 (0.5–1.7)	11/17	2.2 (0.7–6.4)	1.2 (0.7–2.0)
p-Value for trend test		0.85		0.16	0.45
Years since stopped					
4 or more	11/12	1.4 (0.4–4.7)	5/0	–	2.5 (0.9–7.0)
1–3	20/7	2.9 (0.9–9.0)	2/1	10.8 (0.7–166.7)	3.8 (1.3–10.7)
Never stopped or stopped < 1 year ago	56/132	0.6 (0.3–0.9)	15/35	1.4 (0.6–3.5)	0.7 (0.5–1.1)
p-Value for trend test		0.07		0.36	0.33
Dilution					
Diluted	57/80	1.0 (0.6–1.8)	18/23	2.7 (1.1–7.0)	1.3 (0.8–2.1)
Full-strength or both	30/71	0.5 (0.3–1.0)	4/14	1.1 (0.3–4.4)	0.6 (0.3–1.1)
p-Value for trend test		0.07		0.30	0.30
Time in mouth					
Just swish	47/78	1.0 (0.5–1.7)	17/21	2.5 (1.0–6.5)	1.3 (0.8–2.1)
Longer than swish	37/61	0.8 (0.5–1.6)	5/12	2.1 (0.5–8.3)	1.0 (0.6–1.7)
p-Value for trend test		0.61		0.24	0.99
Gargle					
No	18/38	0.6 (0.3–1.3)	8/10	2.9 (0.8–10.6)	0.9 (0.5–1.7)
Yes	69/114	0.8 (0.5–1.3)	14/27	1.9 (0.7–4.9)	1.0 (0.7–1.6)
p-Value for trend test		0.37		0.15	1.00
Rinse					
No	22/54	0.5 (0.2–0.9)	10/16	2.5 (0.8–7.5)	0.8 (0.4–1.4)
Yes	65/98	1.0 (0.6–1.6)	12/21	1.9 (0.7–5.2)	1.1 (0.7–1.7)
p-Value for trend test		0.60		0.16	0.84

[a] Adjusted for age, gender (combined gender model only), tobacco, alcohol, raw fruits and vegetables.
[b] May not add to total number of users due to missing responses to questions.
[c] Subjects who never used mouthwash or used only non-alcohol-containing mouthwash form the referent category.

4 or more years in the past and for heavy smokers and drinkers who stopped use up to 3 years in the past; risks were not elevated among continuing mouthwash users.

Females accounted for 68% of the tobacco and alcohol abstainer cases, 34% of the light users, and 9% of the heavy tobacco and alcohol users. Because of the small number of male cases who used neither tobacco nor alcohol, it was not possible to compute odds ratios adjusting for age, gender, and consumption of raw fruits and vegetables. However, the crude mouthwash risks for tobacco and alcohol-abstaining males and females were identical (OR = 2.1).

For the analysis of salivary cancers we excluded one male and one female case who initiated mouthwash use less than 4 years prior to interview. Of the remaining cases, two of 11 males with salivary cancer used mouthwash, as did six of 12 females. All mouthwash users used only mouthwash containing 25% or more alcohol, and the odds ratio associated with mouthwash use was 0.9 (CI = 0.4–2.1).

Table 3. Adjusted odds ratios[a] for mouthwash use by anatomic site of cancer

Mouthwash use	Oral (n = 205)		Pharyngeal (n = 123)	
	No.[b]	OR (95% CI)[c]	No.[b]	OR (95% CI)[c]
Ever used				
No	129	1.0 (referent)	90	1.0 (referent)
Yes	76	1.1 (0.7–1.8)	33	0.7 (0.4–1.3)
No. times per day				
Less than 2	37	1.2 (0.7–2.1)	19	1.0 (0.5–2.0)
2 or more	39	1.1 (0.6–1.9)	14	0.5 (0.2–1.2)
p-Value for trend test		0.65		0.15
Years used				
0–19	33	0.8 (0.5–1.5)	14	0.6 (0.3–1.3)
20 or more	39	1.5 (0.8–2.6)	18	0.8 (0.4–1.8)
p-Value for trend test		0.21		0.54
Mouthwash-years				
1–29	31	0.9 (0.5–1.6)	19	0.9 (0.4–1.8)
30 or more	39	1.4 (0.8–2.5)	13	0.7 (0.3–1.5)
p-Value for trend test		0.20		0.33
Years since stopped				
4 or more	9	3.3 (1.0–10.6)	7	2.1 (0.6–8.2)
1–3	15	2.8 (0.9–8.4)	7	4.7 (1.1–19.4)
Never stopped or stopped < 1 year ago	52	0.9 (0.5–1.4)	19	0.4 (0.2–0.9)
p-Value for trend test		0.87		0.06
Dilution				
Diluted	55	1.6 (0.9–2.6)	20	0.9 (0.4–1.9)
Full strength or both	21	0.6 (0.3–1.3)	13	0.5 (0.2–1.2)
p-Value for trend test		0.65		0.16
Time in mouth				
Just swish	42	1.4 (0.8–2.3)	22	1.1 (0.5–2.1)
Longer than a swish	26	1.2 (0.7–2.3)	11	0.7 (0.3–1.7)
p-Value for trend test		0.58		0.53
Gargle				
No	19	1.1 (0.5–2.4)	7	0.4 (0.1–1.1)
Yes	57	1.1 (0.7–1.8)	26	0.9 (0.5–1.7)
p-Value for trend test		0.60		0.53
Rinse				
No	28	1.1 (0.6–2.0)	4	0.2 (0.1–0.7)
Yes	48	1.2 (0.7–1.9)	29	1.1 (0.6–2.2)
p-Value for trend test		0.57		0.88

[a] Adjusted for age, gender, tobacco, alcohol, raw fruits and vegetables.

[b] May not add to total number of users due to missing responses to questions.

[c] Subjects who never used mouthwash or used only non-alcohol-containing mouthwash form the referent category.

The odds ratios for mouthwash use were not elevated among persons with any of the three genotypes for alcohol dehydrogenase type 3. The unadjusted odds ratio for the uncommon slow-metabolizing 2-2 genotype was 0.2 (0.0–1.2), while the odds ratio for the other two genotypes, 1-2 and 1-1, were 1.1 (CI = 0.5–2.3) and 0.9 (CI = 0.4–1.9), respectively.

Discussion

Our case–control study in Puerto Rico revealed no overall association between regular use of mouthwash and oral/pharyngeal cancer. Moreover, there were no increased risks with greater exposure to mouthwash, as measured by frequency and/or duration of use, or by other characteristics, such as using undiluted mouthwash, in contrast to findings from the large-scale population-based study on the US mainland [8]. The risk associated with mouthwash use was elevated non-significantly in the small group of persons who used neither tobacco nor alcohol, while no excess risk was seen in light or heavy cigarette smokers and alcohol drinkers. Among smokers and drinkers, the only elevated risks associated with mouthwash were confined to former users.

Prior to our study, eight case–control investigations examined the association between mouthwash use and oral cancer [8, 20–25] or oral dysplasia [42]. Five studies were hospital-based [20, 21, 23–25], one also included subjects identified from death certificates [22], one was population-based [8], and one identified oral dysplasia and control patients through pathology departments [42]. Methodologies and analytic approaches varied considerably. The odds ratios for mouthwash use in the present study (OR = 1.0) fell within the ranges (0.8–2.5) observed in other studies, although the estimates in some studies were based on limited data [26]. In the present study, mouthwash risks for men and women who neither smoked cigarettes nor drank alcohol were the same (OR = 2.1), indicating no gender-specific differences in risk. This is consistent with previous studies [8, 21, 23, 24] in which mouthwash risks for females who did not smoke or drink (ORs from 0.4 to 3.2) were in the same general range as for males (ORs from 0.2 to 2.6).

Dose–response relationships were examined in four case–control studies of oral cancer [8, 21, 22, 25], with three [21, 22, 25] showing no indication of a positive dose–response gradient with greater use of mouthwash. This finding contrasts with the population-based study on the US mainland [8], which showed increasing risks with duration and frequency of use, especially among persons who used mouthwash with the highest concentrations of alcohol (25% or more). Although smaller in sample size (342 cases), the present study used many of the same methods as our previous study (866 cases), including population-based cases, population-based and list-frame sampling for controls, and nearly identical questionnaires.

In this study population, substantially elevated risks of oral cancer were associated with cigarette smoking and

Table 4. Adjusted odds ratios[a] for mouthwash use by cigarette smoking and alcohol drinking behaviors

Mouthwash use	Non-users of tobacco[b] and alcohol		Light cigarette smokers and/or light alcohol drinkers		Heavy cigarette smokers and/or alcohol drinkers	
	No. of cases/ controls[c]	OR (95% CI)[c]	No. of cases/ controls[c]	OR (95% CI)[d]	No. of cases/ controls[c]	OR (95% CI)[d]
Ever used						
No	7/49	1.0 (referent)	27/111	1.0 (referent)	184/141	1.0 (referent)
Yes	12/42	2.8 (0.8–9.9)	14/67	0.8 (0.4–1.7)	83/77	0.9 (0.6–1.3)
No. times per day						
Less than 2	7/19	3.4 (0.8–14.5)	6/31	0.9 (0.3–2.5)	43/36	1.0 (0.6–1.6)
2 or more	5/23	2.3 (0.5–11.1)	8/36	0.8 (0.3–2.0)	40/40	0.8 (0.5–1.3)
p-Value for trend test		0.19		0.55		0.36
Years used						
Less than 20	6/17	6.1 (1.1–33.9)	4/33	0.6 (0.2–2.1)	37/39	0.7 (0.4–1.2)
20 or more	5/20	2.4 (0.5–12.5)	9/33	0.9 (0.3–2.2)	43/31	1.2 (0.7–2.0)
p-Value for trend test		0.32		0.70		0.67
Mouthwash-years						
1–29	6/17	3.7 (0.7–18.3)	4/34	0.6 (0.2–2.1)	40/37	0.8 (0.5–1.4)
30 or more	6/20	5.1 (1.0–26.0)	9/32	0.8 (0.3–2.0)	37/32	1.0 (0.6–1.6)
p-Value for trend test		0.06		0.61		0.82
Years since stopped						
4 or more	1/0	–	3/2	7.6 (1.1–52.7)	12/10	1.1 (0.4–2.7)
1–3	2/1	9.3 (0.6–148.7)	2/3	3.0 (0.4–21.7)	18/4	3.9 (1.3–12.1)
Never stopped or stopped <1 year ago	9/41	2.0 (0.5–8.0)	9/62	0.5 (0.2–1.2)	53/61	0.7 (0.4–1.1)
p-Value for trend test		0.26		0.20		0.25
Dilution						
Diluted	10/23	4.1 (1.0–16.4)	10/38	1.0 (0.4–2.4)	55/39	0.6 (0.3–1.0)
Full-strength or both	2/19	1.4 (0.2–8.7)	4/29	0.5 (0.2–1.8)	28/37	1.2 (0.7–1.9)
p-Value for trend test		0.32		0.38		0.16
Time in mouth						
Just swish	9/19	5.4 (1.2–23.9)	9/38	0.8 (0.3–2.1)	46/41	0.9 (0.6–1.5)
Longer than just swish	3/21	1.8 (0.3–10.2)	4/24	0.8 (0.2–2.6)	35/26	1.2 (0.7–2.1)
p-Value for trend test		0.44		0.75		0.44
Gargle						
No	4/16	2.7 (0.6–13.4)	2/12	0.5 (0.1–2.8)	20/20	0.9 (0.5–1.8)
Yes	8/26	2.9 (0.7–12.0)	12/55	0.9 (0.4–2.0)	63/57	0.8 (0.5–1.3)
p-Value for trend test		0.12		0.68		0.44
Rinse						
No	7/16	3.6 (0.8–15.8)	3/21	0.4 (0.1–1.7)	22/32	0.5 (0.3–1.0)
Yes	5/26	2.1 (0.4–10.4)	11/46	1.0 (0.4–2.3)	61/45	1.1 (0.7–1.8)
p-Value for trend test		0.21		0.87		0.94

[a] Adjusted for age, gender, raw fruits and vegetables.
[b] Non-users of cigarettes, cigars, pipes, and smokeless tobacco.
[c] May not add to total number of users due to missing responses to questions.
[d] Subjects who never used mouthwash or used only non-alcohol-containing mouthwash form the referent category.

alcohol drinking of greater than 21 drinks per week [7]. Among persons with these major risk factors, any additional small risk imparted by alcohol-containing mouthwash might be difficult to detect. Such an effect, however, would more likely be evident among non-users of tobacco and alcohol. Although not statistically significant, the relationship we found between mouthwash use and oral cancer in the small subgroup of non-tobacco users and non-drinkers is consistent with this theory.

Many commercial mouthwashes contain alcohol in the range of 10–25% [36, 43, 44], and heavy users of mouthwash may expose oral mucosal tissues to levels of alcohol in alcoholic beverages that have been shown to induce oral cancer. A single mouthful of mouthwash is unlikely to exceed 2–3 ounces, so that the oral cavity may be exposed to about 0.5–0.75 ounces of alcohol per use (assuming a 25% ethanol content). Hard liquor contains ethanol in the range of roughly 40–50%. Thus, the

ethanol exposure to the oral mucosa from drinking an alcoholic beverage made from 1.5 ounces of hard liquor (assuming a 50% ethanol content) is comparable to that from twice-daily use of 1.5 ounces of mouthwash containing 25% ethanol. These are approximations only, and there may be wide variability between alcoholic beverages and alcohol-containing mouthwash in the amounts of ethanol in contact with the oral mucosa and in many other indicators of exposure. It is noteworthy that the risks of oral cancer associated with alcohol seem to be most pronounced for tumors arising from the floor of the mouth, base of the tongue, and other subsites in greater contact with alcohol and where fluids pool [45, 46]. In our study, mouthwash-related risks for tongue and floor of mouth sites were higher than for most other oral sites, but were not statistically significant.

The potential for type I error (*i.e.*, falsely rejecting the null hypothesis) or bias must be considered in any epidemiologic study. We made multiple comparisons, and statistically significant findings can be expected to occur 5% of the time by chance alone. Selection bias arising from the methods used to ascertain cases and controls does not seem likely in our study. Our use of a population-based cancer registry for case identification, an area probability sampling frame for younger controls, and sampling from Health Care Financing Administration rosters for older controls was likely to yield cases and controls who were highly representative of the island population. The response rate to the interview was good: 69% for male and 77% for female cases, and 82–83% for male and female controls. Eighteen percent of cases but only 2% of controls were not interviewed due to illness, accounting for the major differences in response rates for cases and controls. The percentages of refusals were 5% for cases and 8% for controls. The remaining non-respondents could not be located. There is no obvious reason why mouthwash use would be related to these reasons for non-response.

Potential misclassification of mouthwash use is more problematic. It has been suggested by Shapiro *et al.* [27] that mouthwash use might erroneously seem to be a risk factor for oral cancer if alcohol and tobacco exposures are under-ascertained. In evaluating the validity of recall of tobacco and alcohol use, some studies have found that use may be under-reported [47] or even over-reported [48], while others show fairly good accuracy of recall [49–53]. In our study it was not possible to evaluate the accuracy of reporting of tobacco, alcohol, or mouthwash use.

Oral symptoms associated with the early stages of oral cancer may cause people to start or to quit using mouthwash. Only 6% of mouthwash users indicated "medical" rather than "personal" reasons for use. In addition, no excess risks were observed in subjects who reported initiating mouthwash use less than 4 years before the interview. However, heavy cigarette smokers and alcohol drinkers who quit using mouthwash prior to diagnosis had higher risks than those who continued use, probably due in part to oral cancer symptoms or health provider recommendations.

The lack of consistent findings among studies of mouthwash use and oral cancer may be related to variations in methodologies, limited sample sizes, difficulties in measuring exposures, and low levels of risk. It is also possible that tobacco use and alcohol drinking are differentially under-reported or that mouthwash use is correlated with an unknown risk factor among non-smokers and non-drinkers. Although these potential biases may lead to an elevated odds ratio for mouthwash use in the absence of a true effect, our finding suggesting a mouthwash-related risk among non-smokers and non-drinkers deserves further epidemiologic study using improved methods for validating reports of mouthwash use, tobacco use, and alcohol drinking. In further epidemiologic studies of oral cancer the use of supplementary interviews with other family members would be helpful in evaluating the accuracy of reported behaviors.

We found higher risks of oral cancer among heavy alcoholic beverage drinkers who were homozygous for the 1-1 genotype of alcohol dehydrogenase type 3 [33]. Although mouthwash-related risks were not elevated among those with either the faster or the slower metabolizing genotypes at this locus, future studies of oral cancer should incorporate metabolic susceptibility genes whenever possible. The use of genetic or molecular biomarkers of alcohol or tobacco metabolism may help not only to clarify the mechanisms of alcohol- and tobacco-related carcinogenesis, but also the potential risks associated with alcohol-containing mouthwash.

Mouthwash use is a very common behavior. The percentage of male (40%) and female (44%) controls using mouthwash in Puerto Rico was nearly the same as in the larger population-based study on the US mainland (44% of male and 45% of female controls) [8]. However, in Puerto Rico, 54% of controls who used mouthwash took it twice or more per day, while only 23% of mouthwash-using mainland controls reported similar levels of use [8]. Despite the widespread use of mouthwash in Puerto Rico, the attributable risk for mouthwash use is likely to be small, since no excess risk was detected among (1) heavy smokers or drinkers who comprise the majority of oral cancer patients, or (2) the large subgroup of current mouthwash users.

In conclusion, our population-based case–control study of oral cancer in Puerto Rico yielded no evidence of an overall excess risk associated with use of

mouthwash. However, excess risks were observed among those who neither smoked cigarettes nor drank alcohol, the subgroup in which any effect of alcohol-containing mouthwash should be most evident. Larger studies are needed to better examine the effects of mouthwash in population subgroups hypothesized to be at greatest risk. Further research is needed to improve the assessment of mouthwash exposure to understand the patterns of mouthwash use among persons with early oral cancer symptoms and to clarify the potential effects of alcohol-containing mouthwash in the etiology of oral cancer.

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